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# Potential of *Beauveria bassiana* and *Metarhizium anisopliae* as Biological Control Agents against the Destructive Pests *Callosobruchus maculatus* and *Myzus persicae*

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**Abstract**
In the present investigation, the entomopathogenic fungi *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff) were subjected to evaluation under controlled laboratory conditions in order to determine their insecticidal efficacy. The pathogenicity of these fungi was tested against the green aphid *Myzus persicae* (Hemiptera: Aphididae) and the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Chrysomelidae). Both *B. bassiana* and *M. anisopliae* exhibited pathogenic activity at all tested concentrations ($1 \times 10^6$, $1 \times 10^7$, and $1 \times 10^8$ spores/ml) against *M. persicae*. The highest concentration ($1 \times 10^8$ spores/ml) of *B. bassiana* produced the most pronounced effect, reducing populations by 82% in *M. persicae* and 77.4% in *C. maculatus* within seven days. The two fungal isolates were tested against adults of *Callosobruchus maculatus* (F.). The insects were exposed to aqueous suspensions at varying concentrations, with untreated cowpea weevils serving as the control group. Probit analysis revealed that the $LC_{50}$ values were $1.29 \times 10^7$ spores/ml for *Beauveria bassiana* and $6.67 \times 10^7$ spores/ml for *Metarhizium anisopliae*. In conclusion, the application of *B. bassiana* and *M. anisopliae* as biopesticides represents a sustainable and environmentally friendly strategy for the management of insect pests in agriculture.

**Keywords:** Beauveria bassiana, metarhizium anisopliae, myzus persicae, callosobruchus maculatus, biological control

## Introduction
Aphids are among the most destructive pests affecting crop production, particularly in crops such as pepper, cucumber, and eggplant. The prolonged and intensive application of synthetic insecticides to manage the green aphid, *Myzus persicae* (Hemiptera: Aphididae), in agricultural systems worldwide has led to the development of pest resistance and contributed to environmental pollution. Consequently, the significance of entomopathogenic fungi as alternative agents for pest control is increasingly being recognized. Leguminous crops constitute a critical component of human nutrition owing to their elevated levels of proteins, lipids, and carbohydrates, in addition to their contribution to soil fertility through nitrogen fixation. However, their cultivation and storage are frequently compromised by seed beetles, which inflict substantial postharvest losses. Among these, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is recognized as one of the most destructive pests of legumes, with a global distribution across tropical and subtropical regions. Under storage conditions, this species exhibits rapid population growth, completing a generation approximately every month (Ouedraogo *et al*., 1996) [15]. Consequently, effective management strategies are imperative to mitigate grain losses (Tapondjou *et al*., 2002) [22].

Growing public concern regarding environmental safety has stimulated research into alternative pest control methods (Inglis *et al*., 1997; Lomer *et al*., 2001) [12]. Biological control, particularly the application of entomopathogenic fungi and other microbial agents, has emerged as a promising approach to reduce reliance on synthetic insecticides. Although the entomopathogenic potential of fungi against insect pests has been investigated for decades, their application in the management of storage pests remains comparatively underexplored (Khan and Selman, 1988; Rodrigues and Pratissoli, 1990; Adane, 1994; Adane *et al*., 1996; Padín *et al*., 1997 [2, 3, 11, 17, 19]; Hidalgo *et al*., 1998; Moino *et al*., 1998).

## Materials and methods
### Rearing of tested insects
Before beginning the experiments, cowkpeas were kept at 20 °C for two weeks to eliminate of other pests. The moisture content of cowpea seeds was determined to be 10.9% using a Grain Moisture Meter Wile 55 (Farm comp Oy, Finland). Adults of *Callosobruchus maculatus* were introduced into 500 ml glass jars, each containing 200 g of sterilized cowpea (*Cicer arietinum* L. var. Kocbasi) seeds. The jars were covered with cloth to permit aeration and maintained under controlled environmental conditions of 25±1 C, relative humidity of 65±5%, and a photoperiod of 16:8 h (light: dark) to facilitate oviposition. The cultures were sieved daily to separate and record the populations of male and female adults (Ozdemir *et al*., 2020) [16]. The aphid *Myzus persicae* was collected from okra (*Abelmoschus esculentus*) plants located within the premises of the Faculty of Agriculture, Cairo University, Giza Governorate.

### Bioassay procedure
The concentrations of *Beauveria bassiana* and *Metarhizium anisopliae* were prepared at $1 \times 10^8$, $1 \times 10^7$, and $1 \times 10^6$ spores/ml. The activity of both fungal species requires high humidity, while exposure to solar radiation significantly reduces spore viability due to increased mortality. Fungal applications are most effective when administered under cool temperature conditions. Insect mortality is influenced by several factors, including the viability and density of spores contacting the insect, host susceptibility, insect age, and prevailing environmental conditions. Both fungal species, at the three tested concentrations, were evaluated against the green aphid (*Myzus persicae*) and the cowpea weevil (*Callosobruchus maculatus*).

**Treating of green aphid**

Each treatment consisted of ten leaves of green pepper (*Capsicum annuum*), serving as ten replicates. Ten aphid individuals were introduced onto each leaf (one replicate). The leaves were then sprayed using a glass atomizer positioned 30 cm above the surface, applying 1 ml of spore suspension per leaf. Three concentrations of spore suspensions ($1 \times 10^6$, $1 \times 10^7$, and $1 \times 10^8$ spores/ml) were prepared in 0.1% Triton X-100, which was used as a surfactant. The entomopathogenic fungal isolates *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff) were procured from the Bio-Insecticides Production Unit of the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. The spray method was employed to assess the virulence of the fungi (Hassan *et al*., 2023) [9].

**Treating of cowpea weevil**

The treated insects were subsequently transferred into plastic plates (8.8 cm diameter, 60.7 $cm^2$) containing sterile filter paper (8.8 cm diameter) and 20 g of cowpea seeds for *Callosobruchus maculatus*. The plates were sealed with Parafilm to prevent insect escape, with three replicates established for each treatment. The insects were maintained in an incubator under controlled conditions of $27 \pm 2$ °C and $65 \pm 5$% relative humidity. The total number of living and dead adults was recorded over a period of nine days (Cherry, 2005) [5]. To monitor fungal outgrowth, dead insects from each plate were rinsed in sterile distilled water and subsequently transferred to new plates maintained at high relative humidity. The mortality percentages were corrected using Abbott's formula (Abbott, 1925) [1]. The $LC_{50}$, $LC_{90}$, and slope values were calculated following the method described by Finney (1971) [6], employing the "Ldp Line" software developed by Bakr (2000) [4].

**The determination of Glutathione S-transferase (GST)**

The conjugate, S-(2,4-dinitrophenyl)-L-glutathione, was detected following the method described by Habig *et al*. (1974) [7].

**Statistics**

Lethal concentration values ($LC_{50}$ and $LC_{90}$) and slope parameters were estimated following the probit analysis method described by Finney (1971) [6], employing the *Ldp Line* software (Bakr, 2005). Each experimental treatment was replicated up to five times. The resulting data were subjected to one-way analysis of variance (ANOVA) using SPSS statistical software, version 17.0. When significant differences were detected ($P < 0.05$), mean comparisons were performed using Duncan's multiple range test.

**Results and discussion**

**The effect of entomopathogenic fungi on the green peach aphid (*Myzus persicae*)**

The susceptibility of adult individuals to isolates of *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff)was determined. Mortality percentages following exposure to spore suspensions at concentrations of $1 \times 10^6$, $1 \times 10^7$, and $1 \times 10^8$ spores/ml were recorded over a seven-day period, as presented in Fig. 1. The results indicated a gradual increase in mortality with rising spore concentrations. At the lowest concentration ($1 \times 10^6$ spores/ml), mortality rates were 53.33% and 50.96% for *B. bassiana* and *M. anisopliae*, respectively, after seven days. In contrast, the highest concentration ($1 \times 10^8$ spores/ml) resulted in mortality rates of 82% and 80% for the respective isolates over the same period. The results demonstrated that *Beauveria bassiana* was more effective against adult green aphids compared with *Metarhizium anisopliae*, as indicated by the $LC_{50}$ values. The $LC_{50}$ value for *B. bassiana* was $1.412 \times 10^7$ spores/ml, whereas that for *M. anisopliae* was $2.14 \times 10^7$ spores/ml, as presented in Fig. 1 and Table 1.

The results obtained in the present study are consistent with those reported by various researchers worldwide. These findings are in agreement with Saranya *et al*. (2010) [21], who investigated the pathogenicity of five entomopathogenic fungi—*Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Hirsutella thompsonii*, and *Cladosporium oxysporum*—against adults of *Aphis craccivora* under laboratory conditions. Similarly, the present results align with the laboratory bioassay studies conducted by Sahar *et al*. (2016) [20], in which four different concentrations ($1 \times 10^6$, $1 \times 10^7$, $1 \times 10^8$, and $1 \times 10^9$ spores/ml) of *B. bassiana*, *M. anisopliae*, *Paecilomyces lilacinus*, and *Lecanicillium antillanum* were tested

**Table 1:** Comparative between two isolates of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against green aphid adult stage according to $LC_{50}$. (spores/ml).

| Line name | LC50 spores/ml | Lower limit | Upper limit | Index | RR | Slope |
|---|---|---|---|---|---|---|
| *B.bassiana* | $1.412 \times 10^7$ | 1030600 | 41784000 | 100.000 | 0.658 | 0.435 |
| *M. anisoplae* | $2.14 \times 10^7$ | 5098500 | 48899000 | 65.778 | 1.000 | 0.420 |

**Treating of cowpea weevil**

The mortality percentages of *Callosobruchus maculatus* adults after seven days of exposure to different spore concentrations are presented in Figure 2. Mortality rates of 55.1% and 77.4% were achieved with *Beauveria bassiana* at concentrations of $1 \times 10^7$ and $1 \times 10^8$ spores/ml, respectively. The $LC_{50}$ and $LC_{90}$ values for *B. bassiana* were $1.29 \times 10^7$ and $2.57 \times 10^9$ spores/ml, respectively. In the case of *Metarhizium anisopliae*, the $LC_{50}$ and $LC_{90}$ values were $6.67 \times 10^7$ and $62.88 \times 10^9$ spores/ml, respectively, after seven days of treatment. As the exposure period increased, both $LC_{50}$ and $LC_{90}$ values decreased. genetic background. Additionally, host physiology and morphology affect susceptibility to biological control agents such as entomopathogenic fungi (Fargues *et al*., 1996) [24]. Therefore, differences in the susceptibility of storage beetles to *B. bassiana* cannot be explained solely by the concentration of conidia applied (Cox *et al*., 2004) [23]. The findings of the present study further support those of Cherry *et al*. (2005) [5], who reported that different isolates of *M. anisopliae* and *B. bassiana* can effectively control *C. maculatus* through immersion bioassays. A comparative evaluation was conducted between two isolates of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against the adult stage of *Callosobruchus maculatus*, based on $LC_{50}$ and $LC_{90}$ values.

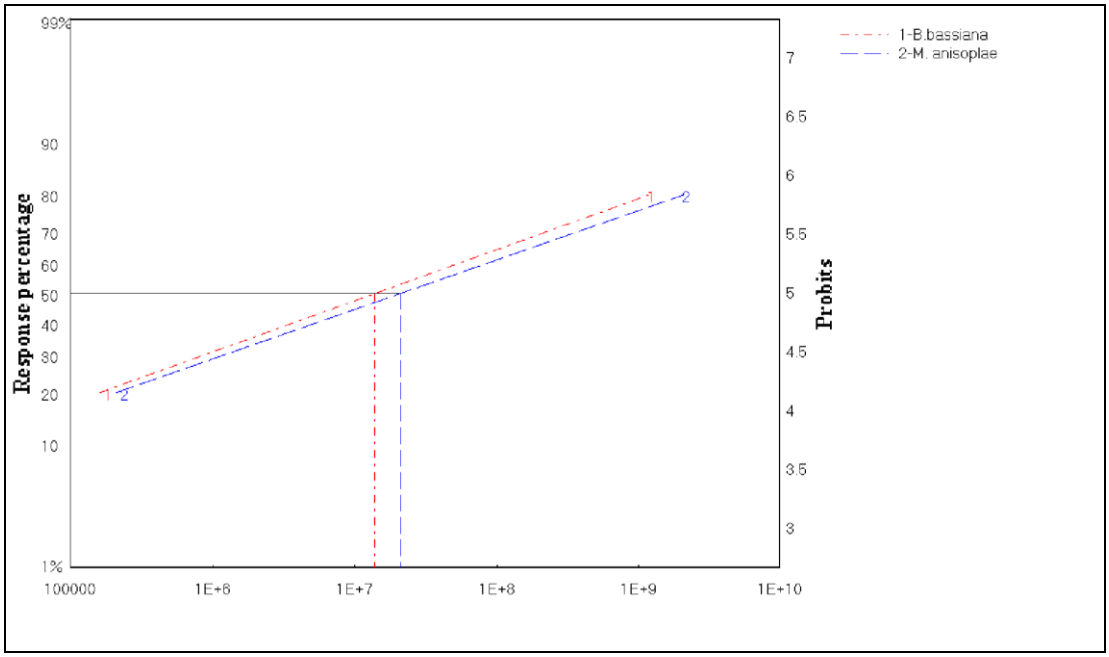
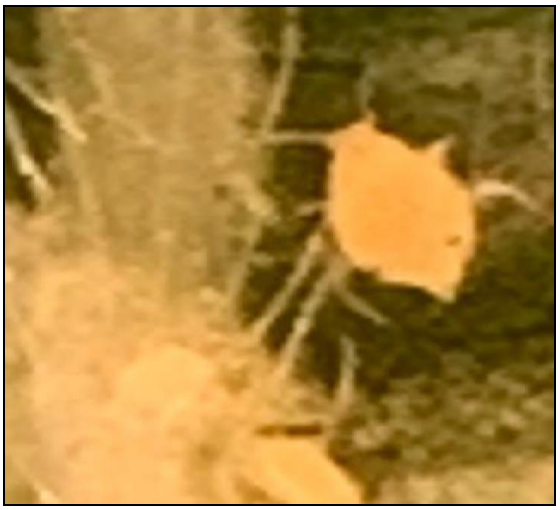
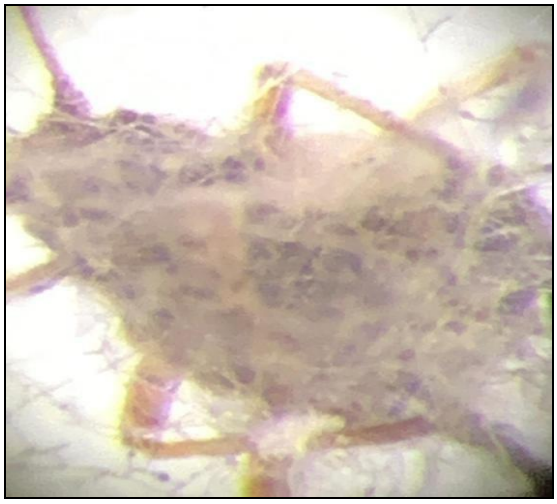



**Fig 1:** LD-P lines of the two entomopathogenic fungi against the green aphid



**Untreated green aphid adult**



**Treated green aphid adult with Beauveria bassiana**

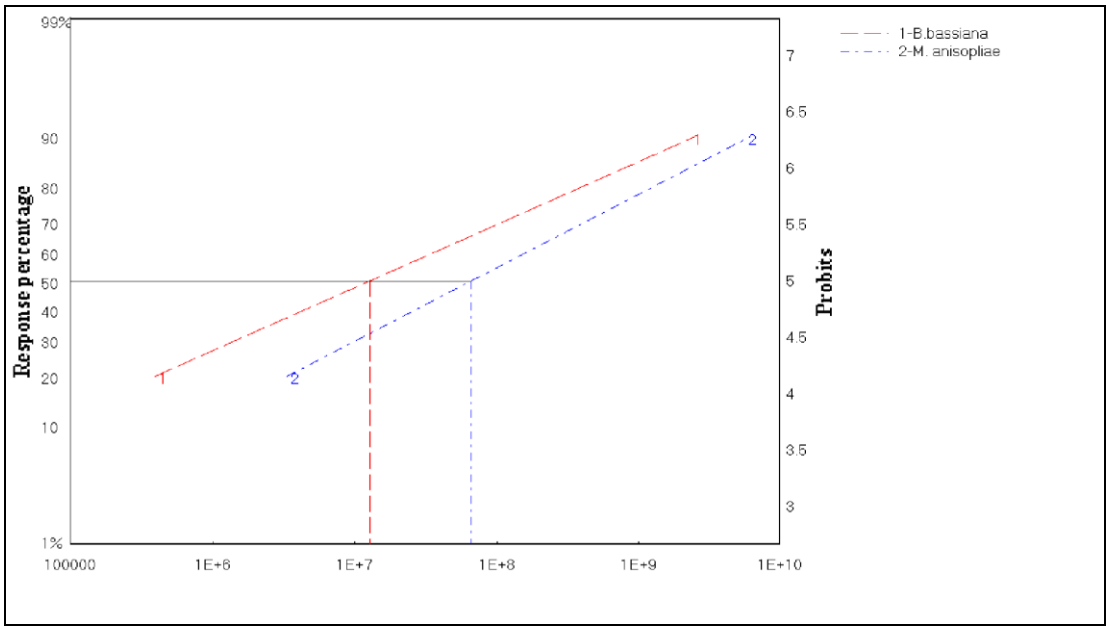


**Fig 2:** LD-P lines of the two entomopathogenic fungi against the cowpea weevil

| Line name | $LC_{50}$ spores/ml | Lower limit | Upper limit | Index | RR | Slope | LC90 spores/ml |
|---|---|---|---|---|---|---|---|
| *B.bassiana* | $1.29\times10^{7}$ | 5751400 | 22704000 | 100.000 | 0.194 | 0.557 | $2.5710\times10^{9}$ |
| *M. anisopliae* | $6.67\times10^{7}$ | 44199000 | 97626000 | 19.392 | 1.000 | 0.649 | $62.88\times10^{9}$ |

**Table 3:** The effect of *Beauveria bassiana* ($LC_{50}$) on glutathione-S-transferase activity

| Sample | | m mole/g.tissue | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | Mean ± SD |
| control | 8.1 | 7.9 | 8.33 | 8.11 ± 2a |
| 24 hr. | 9.50 | 9.70 | 10 | 9.7 ± 2b |
| 48 hr. | 10.13 | 9.80 | 11.1 | 10.34 ± 6c |
| 72 hr. | 8.35 | 8.60 | 8.49 | 8.48 ± 0.1a |

A mean followed by the same letters with in the same column are not significantly different ($p \geq 0.05$)

Treated Cowpae weevil adult with Beauveria bassiana

**Effect of *Beauveria bassiana* on Glutathione - S transferase**

The values of total GST extracted from the homogenate of green aphid adults infected with *Beauveria bassiana* are presented in Table 3. The results indicated no significant differences between treated and untreated aphids at 24 and 48 hours post-treatment. However, a significant difference was observed after 72 hours, as shown in Figure 3.

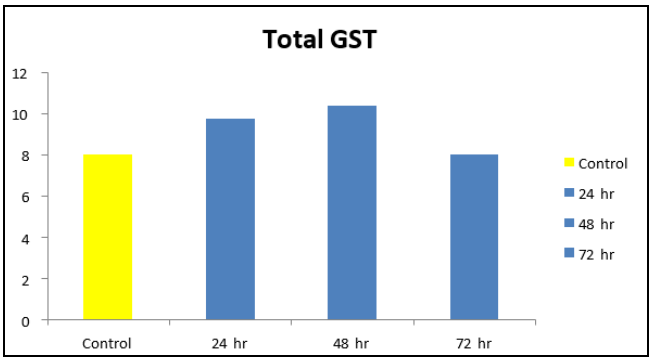



**Fig 3:** The effect of *Beauveria bassiana* ($LC_{50}$) on glutathione-S-transferase activity

*Beauveria bassiana* exhibited control activity against the green aphid *Myzus persicae*, with the detoxification enzyme GST measured in aphids treated at the $LC_{50}$ concentration ($1.412 \times 10^{7}$ spores/ml). After 24 hours of treatment, a significant increase in GST activity was observed compared to the control, indicating its involvement in the detoxification process. However, after 72 hours, enzyme activity decreased relative to the control, suggesting that aphid mortality resulted from the inability to counteract the fungal infection. These findings are consistent with the observations of Nauen *et al*. (2002) [14].

Evidence from the experiments further demonstrated that *B. bassiana* significantly reduced populations of both *Callosobruchus maculatus* and *M. persicae*. A comparison of $LC_{50}$ values and related results clearly indicated that adult *M. persicae* were more sensitive to *B. bassiana* than *C. maculatus*.

In conclusion, microbial pest control represents a safe and non-chemical alternative that can provide environmental benefits while ensuring more effective protection of plants.

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